Certificate of Facsimile

I hereby certify that this paper is being transmitted by facsimile to the Patent and Trademark Office at (703) 308-4556 on the date shown below:

By:_____

Date:_____

Atty Docket No.: 2001.689 US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

SHEPARD, SCOT

Serial No.: 09/855,634 Group Art Unit: 1617

Filed: May 14, 2001 Examiner: Bahar, M.

For: METHODS AND COMPOSITIONS FOR INACTIVATING VIRUSES

DECLARATION OF SCOT R. SHEPARD

1. I, Scot R. Shepard, residing in North Carolina, state that the following is true and correct to the best of my knowledge, under penalty of perjury. These statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 USC and that such willful false statement may jeopardize the validity of the instant patent application or any patent issuing thereon.

2. I am an Associate Director of Process Development for Diosynth RTP, Inc. Diosynth RTP is a company of Akzo Nobel NV, the assignee of record. My office is located in Cary, North Carolina, USA.

3. My educational background includes an MS in Biology from Florida Atlantic University.

4. I am the sole inventor of application serial number 09/855,634, titled Methods and Compositions for Inactivating Viruses.

5. One of the issues I encountered in the prior art was the inactivation of viral contaminants in a biological source material or process intermediate. My solution was to contact the biological source material or process intermediate with an alkylamine. Namely, an alkylamine selected from the group consisting of dimethyldecylamine, dimethyltridecylamine, dimethylundecylamine, dimethyldidecylamine, dimethyltetradecylamine, dimethylhexadecylamine, dimethyldecylamineoxide, dimethylundecylamineoxide,

dimethyldidecylamine, dimethyltetradecylamine, dimethylhexadecylamine, dimethyldecylamineoxide, dimethylundecylamineoxide, dimethyldidecylamineoxide, dimethytetradecylamineoxide and dimethyltridecylamineoxide, a class of chemicals structurally distant from the polyoxyethylene based non-ionic detergents enumerated in '189. Glycerol was also included in some embodiments of my invention because previous work in my laboratory demonstrated that glycerol enhances the kinetics of alkylamine induced membrane disruption. Whereas it is generally understood in the art that the solvent-detergent method taught by Prince uses said combination to enhance aqueous solubility.

9. The claims made in '189 teach the use of non-ionic detergents and the text includes the very broad mention of non-anionic detergents. The definition of a detergent - a synthetic cleansing agent resembling soap in the ability to emulsify oil and hold dirt, and containing surfactants which do not precipitate in hard water; may also contain protease enzymes and whitening agents – illustrates the lack of chemical and molecular specificity provided by the term. And, it is known in the art that not all detergents are effective viral inactivators. Thus, one skilled in the art could not reliably predict that a particular chemical or a class of chemicals, that happen to have detergent-like properties, would inactivate viruses simply because Prince showed that polyoxyethylene based surfactants do inactivate viruses. This statement is substantiated by the fact that we have observed that alkylamines with different hydrocarbon chain lengths have variable effects on membrane disruption. For example, certain short chain alkys, which possess detergent-like properties, do not effectively disrupt microbial phospholipid bilyaers *in situ*. Additionally, certain long chain alkyls, which posses detergent-like properties, do not have suitable aqueous solubility to effect membrane disruption in the absence of secondary molecules that enhance solubility of the former. Thus, the premise that the viral inactivation properties of alkylamines are obvious due to the teachings of '189 is completely unfounded.

10. My work on identification of viral inactivators was inspired by our work done on the extraction of soluble cytoplasmic proteins from the yeast *Pichia pastoris*. Our yeast data clearly indicated that the cell membrane of the yeast was compromised by treatment with DMA-C14. The reasoning was that if a reagent could destabilize the yeast (a eukaryotic lipid bilayer) cell membrane then the lipid bilayer of enveloped animal viruses (which are derived from eukaryotic cells) may also be destabilized. And, that destabilization of the viral membrane would render the virus non-infective.

11. It is my opinion that reagents that are not effective for protein release from yeast would not be effective viral inactivators. Toward this end we present the table below. The table gives a list of non-anionic detergents that were screened for the ability to extract proteins from yeast. There are 19 reagents listed. Seven of the reagents did not extract proteins from yeast cells. One of these is from the same chemical class as DMA-C14. A second is from a closely related class (dimethylamine oxides). Three of the Tritons, which are non-ionic polyoxyethylene detergents, were not effective protein extractors. Triton X-100 is one of the molecules most often used in the practice of the '189 patent. Methylamine did not work even though many of the dimethylamines were effective.

12. This is evidence that one can not determine *a priori* that a molecule with detergent-like

properties will be an effective disruptor of a biological membrane.

REAGENT TESTED	ABBREVIATION	EFFECTIVE
N, N, Dimethyloctylamine	DMA C8	NO
N, N, Dimethyldecylamine	DMA C10	YES
N, N, Dimethylundecylamine	DMA C11	YES
N, N, Dimethyltridecylamine	DMA C13	YES
N, N, Dimethyltetradecylamine	DMA C14	YES
N, N, Dimethylhexadecylamine	DMA C16	YES
N, N, Dimethyldecylamine N-oxide	DMO C10	YES
N, N, Dimethylundecylamine N-oxide	DMO C11	YES
N, N, Dimethyldodecylamine N-oxide	DMO C12	YES
N, N, Dimethyltridecylamine N-oxide	DMO C13	YES
N, N, Dimethyltetradecylamine N-oxide	DMO C14	YES
N, N, Dimethyloctadecylamine N-oxide	DMO C18	NO
Triton X-45	Triton X-45	NO
Triton X-100	Triton X-100	YES
Triton X-114	Triton X-114	NO
Triton X-305	Triton X-305	NO
Triton X-405	Triton X-405	YES
Triton X-705	Triton X-705	YES
Didecylmethylamine	MA C10	NO

Date

Scot R. Shepard